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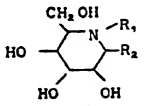
N-alkylated derivatives of 5-amino-5-deoxy-D-glucose, processes for their preparation and their use

The present invention relates to new N-alkylated derivatives of 5-amino-5-deoxy-D-glucose, several processes for their preparation and their use as medicaments, in particular as agents against diabetes, hyperlipaemia and adiposity, and in the nutrition of animals for influencing the lean meat/fat ratio in favour of the proportion of lean meat.

It has now been found that the new N-alkylated derivatives of 5-amino-5-deoxy-D-glucose, of the formula I,



I

in which

R_1 represents an optionally substituted, straight-chain, branched or cyclic saturated or unsaturated aliphatic hydrocarbon radical or an optionally substituted aromatic or heterocyclic radical and

R_2 denotes H, OH, alkoxy, amino, monoalkylamino and dialkylamino, $-SO_3H$ or -CN,

are powerful inhibitors for α-glucosidases, in particular for saccharases. Moreover, these compounds are substances which inhibit the intestinal absorption of glucose.

By optionally substituted radicals R_1 there are preferably understood those hydrocarbon radicals or heterocyclic radical in which one or more hydrogen atoms are replaced by halogen, hydroxyl, alkoxy or aryloxy, or by optionally substituted amino groups, or by optionally substituted aryl or heterocyclic radicals.

R_1 in the meaning of a saturated aliphatic hydrocarbon radical preferably represents straight-chain or branched alkyl

with 1 to 30, in particular 1 to 18, carbon atoms. Examples which may be mentioned are methyl, ethyl, n-propyl, n-butyl, t-butyl, n-hexyl, n-octyl, oct-2-yl, dodecyl, lauryl, cetyl and stearyl.

These alkyl radicals can carry one or more, preferably 1 to 5, identical or different substituents. Examples of substituents which may be mentioned are: hydroxyl, and alkoxy with preferably 1 to 4 carbon atoms, in particular methoxy and ethoxy; amino, and monoalkylamino and dialkylamino with preferably 1 to 4 carbon atoms per alkyl radical, in particular monomethylamino, monoethylamino, dimethylamino and diethylamino; mercapto, and alkylthio with preferably 1 to 4 carbon atoms, in particular methylthio and ethylthio; halogen, preferably fluorine, chlorine and bromine; alkylcarbonyl with preferably 1 to 4 carbon atoms in the alkyl radical; and carboxyl, nitro, cyano, the aldehyde group and the sulphonic acid group.

R_1 in the meaning of an unsaturated hydrocarbon radical preferably represents straight-chain or branched alkenyl radicals with 2 to 6 carbon atoms, which can carry further substituents, such as hydroxyl, alkoxy with 1 to 4 carbon atoms, mercapto, alkylthio with 1 to 4 carbon atoms, halogen and nitro, inter alia.

R_1 in the meaning of a cyclic hydrocarbon radical preferably represents a carbocyclic radical with preferably 3 to 7 carbon atoms, which can be substituted, possible substituents being the groups and atoms mentioned in the case of the open-chain hydrocarbon radicals.

R_1 in the meaning of an aromatic radical preferably represents aromatic radicals with 6 or 10 carbon atoms in the aryl part, in particular phenyl, which can be substituted.

The aryl radicals can carry one or more, preferably 1 to 3, identical or different substituents. Examples of substituents which may be mentioned are: alkyl with 1 to 10 carbon atoms, which in turn can again be substituted, for example by chlorine, nitro or cyano; optionally substituted alkenyl radicals with 1 to 10 carbon atoms; hydroxyl, alkoxy with preferably 1 to 4 carbon atoms; amino, and monoalkylamino and dialkylamino with preferably 1 to 4 carbon atoms per alkyl radical; mercapto, and alkylthio with preferably 1 to 4 carbon atoms; carboxyl, carbalkoxy with preferably 1 to 4 carbon atoms, the sulphonic acid group, alkylsulphonyl with preferably 1 to 4 carbon atoms and arylsulphonyl, preferably phenylsulphonyl; aminosulphonylsulphonyl, and alkylaminosulphonyl and dialkylaminosulphonyl with 1 to 4 carbon atoms per alkyl group, preferably methylaminosulphonyl and dimethylaminosulphonyl; nitro, cyano or the aldehyde group; alkylcarbonylamino with preferably 1 to 4 carbon atoms; and alkylcarbonyl with 1 to 4 carbon atoms, benzoyl, benzylcarbonyl and phenylethylcarbonyl, it being possible for the last-mentioned alkyl, phenyl, benzyl and phenylethyl radicals in turn to be again substituted, for example by chlorine, nitro or hydroxyl.

The heterocyclic radicals R_1 are preferably derived from hetero-paraffinic, hetero-aromatic or hetero-olefinic 5-membered or 6-membered rings with preferably 1 to 3 identical or different hetero-atoms. The hetero-atoms are oxygen, sulphur or nitrogen. These ring systems can carry further substituents, such as, for example, hydroxyl, amino or C_1-C_4-alkyl groups, or benzene nuclei or other, preferably 6-membered, heterocyclic rings of the type mentioned can be fused to them.

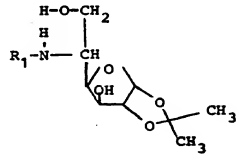
Particularly preferred heterocyclic radicals are

derived, for example, from furane, pyrane, pyrrolidine, piperidine, pyrazole, imidazole, pyrimidine, pyridazine, pyrazine, triazine, pyrrole, pyridine, benzimidazole, quinoline, isoquinoline or purine.

When R_2 denotes alkoxy, monoalkylamino or dialkylamino, each alkyl radical preferably has 1 to 4 C atoms.

In the compounds of the formula I, R_2 preferably represents H, OH, SO_3H and CN. Those compounds of the formula I in which R_2 equals H or OH are particularly preferred.

Furthermore, it has been found that N-alkylated derivatives of the 5-amino-5-deoxy-D-glucose of the formula I are obtained when, in compounds of the formula II



II

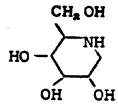
in which

R_1 has the meaning indicated above,

the isopropylidene protective group is removed by careful acid hydrolysis, it sometimes being appropriate to isolate the compounds of the formula I in the form of adducts of sulphurous acid or of hydrocyanic acid (R_2 = SO_3H or CN). The compounds of the formula I in which R_2 = OH are liberated from the bisulphite addition products by treatment with bases, preferably alkaline earth metal hydroxides, such as $Ca(OH)_2$ or $Sr(OH)_2$, but in particular $Ba(OH)_2$. The compounds of the formula I in which R_2 = H are obtained from compounds of the formula I in which R_2 = OH by reaction with hydrogen donor

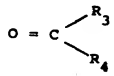
reducing agents, such as, for example, $NaBH_4$.

It has also been found that compounds of the formula I in which R_2 = H are obtained when 1-desoxynojirimycin of the formula III



III

is reacted with carbonyl compounds of the formula IV



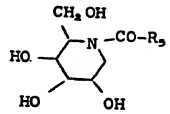
IV

in which

R_3 and R_4 either have H or have the meaning given for R_1 or are members of an alicyclic or heterocyclic ring,

in the presence of a hydrogen donor reducing agent.

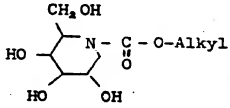
Compounds of the formula I in which R_2 = H are furthermore obtained when amides of the formula V



V

in which

R_5 is either H or has the meaning given for R_1, or carbamates of the formula VI



VI

- optionally also derivatives of these compounds which are provided with hydroxyl-protective groups - are reduced to amines with an amide-reducing agent.

A further process for the preparation of compounds of the formula I in which R_2 = H consists in reacting 1-desoxinojirimycin of the formula III with reactive alkylating agents of the formula VII

$$Z - R_1 \qquad \text{VII}$$

wherein

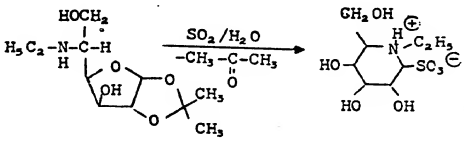
R_1 has the meaning indicated above and

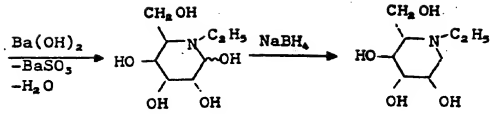
Z is an easily eliminated group, such as, for example, halide or $^{\ominus}O\text{-}SO_3H$, which is customary in alkylating agents.

Because of their strongly pronounced inhibiting action against α-glucosidases, the new derivatives of 5-amines-5-deoxy-D-glucose are valuable agents for influencing a number of metabolism processes and thus enrich the range of medicaments.

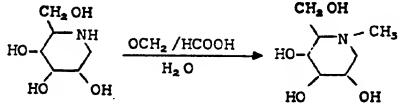
The individual procedures for the preparation of the active compounds according to the invention are illustrated below:

If a compound of the formula II in which R_1 = ethyl is used as the starting material, the course of the reaction can be represented as follows

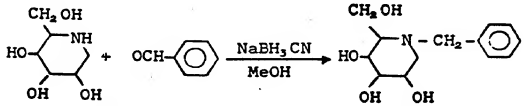




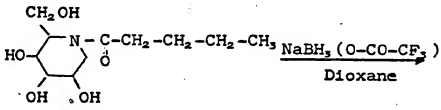
If 1-desoxynojirimycin of the formula III and formaldehyde are used as starting materials, the following equation results:

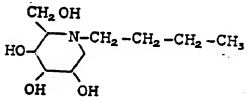


If benzaldehyde is used as the carbonyl component, the reductive alkylation is carried out as follows:

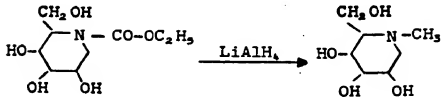


If acid-amides of the formula V are used as starting materials, the reaction can be described as follows:

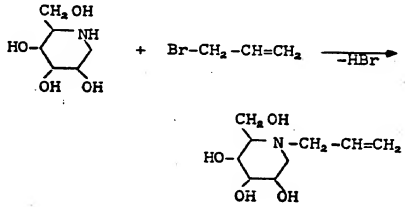




Urethanes of the formula IV, optionally in the form of derivatives provided with hydroxyl-protective groups, can be reduced to N-methyl-1-desoxynojirimycin with $LiAlH_4$:

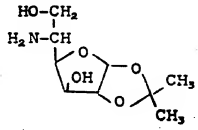


For the reaction of 1-desoxynojirimycin with alkylating agents, the reaction with allyl bromide may be indicated as an example:



The compounds of the formula II used as starting materials are new. However, they can be prepared from compounds which are known from the literature by processes which are in themselves known.

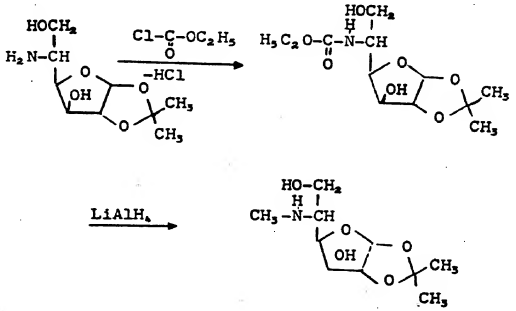
Thus, for example, it is possible to use the compound of the formula VIII, which is known from the literature,



VIII

as a starting material and to react this with carbonyl compounds of the formula IV in the presence of a hydrogen donor reducing agent to give compounds of the formula II.

Furthermore, it is possible to react the compound VIII with reactive acid derivatives to give acid amides or urethanes and to reduce these to amines with an amide-reducing agent. This may be illustrated by an example:

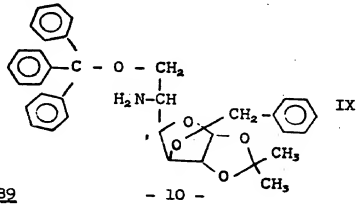


The compound of the formula VIII can also be reacted with reactive alkylating agents of the formula VII

$$Z - R_1 \qquad \text{VII}$$

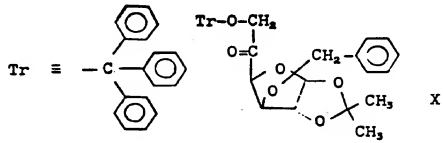
to give compounds of the formula II.

Furthermore, in the abovementioned reactions, instead of the compound VIII it is also possible to employ known partially protected derivatives of the formula IX



and then to remove the trityl and benzyl protective groups in a known way, for example with sodium in liquid ammonia.

To prepare compounds of the formula II, it is also possible to react the compound of the formula X, which is likewise known from the literature,



with amines of the formula XI

$$R_1 - NH_2 \qquad XI$$

in the presence of a hydrogen donor reaction agent, for example in the presence of $NaBH_3CN$. As a rule, a diastereomer mixture is formed in this reaction. The diastereomer which is not desired is appropriately separated off at this stage or at a later stage by the customary chromatographic methods or by fractional crystallisation. Finally, the trityl and benzyl protective group are split off in a known way, for example with sodium in liquid ammonia.

The isopropylidene protective group is split off from the compounds of the formula II in a moderately strongly acid to weakly acid solution, preferably in a pH range between 1 and 4, in aqueous solution or in a water-miscible, water-containing organic solvent. Acids which can be used are dilute minderal acids, such as, for example, sulphuric acid, or also organic acids, such as acetic acid. The reaction is preferably carried out under atmospheric pressure and at a temperature between room temperature and the boiling point of the solvent.

In order to work up the reaction mixture, the acid is neutralised and separated off as a salt or with the aid of a

basic ion exchanger. The isolation of the compounds of the formula I in which R_2 = OH is then appropriately effected by careful removal of the solvent, for example by lyophilisation.

A preferred embodiment of the splitting off of the isopropylidene protective group from compounds of the formula II consists in saturating the aqueous or water-containing alcoholic solution of the compounds of the formula II with SO_2 and storing the saturated solution at temperatures between 20° and 50°C for several days. The compounds of the formula I are then obtained as bisulphite adducts (R_2 = $-SO_3H$), which in most cases readily crystallise, from which the compounds of the formula I can be liberated with the aid of, for example, aqueous $Ba(OH)_2$.

The compounds of the formula I in which R_2 = OH are reduced to compounds of the formula I in which R_2 = H by using alkali metal borohydrides, alkali metal cyanoborohydrides or dialkylaminoboranes. It is preferable to use sodium borohydride in aqueous solution or in a water-miscible water-containing organic solvent, such as, for example, dioxane, at room temperature or optionally elevated temperature.

The starting material of the formula III is known and is obtained either by catalytic hydrogenation of najirimycin, which is obtainable by fermentation [see S. Inoye et al., Tetrahedron 23, 2125-2144 (1968)], or by extraction from mulberry tree bark (see DT-OS (German Published Specification) 2,656,602), or completely synthetically. 1-Desoxynojirimycin can also be prepared by a new advantageous process by cultivating organisms of the Bacillaceae family in customary fermentation vessels in customary nutrient solutions at temperatures from about 15 to about 80°C for about 1 to about 8 days, with aeration, centrifuging off the cells and isolating the desoxy

compounds from the culture broth or the cell extracts by customary purification processes [German Patent Application P 26 58 563.7 - (Le A 17 587)].

The carbonyl compounds of the formula IV are either known or can be prepared by standard processes.

In detail, typical examples which may be mentioned are: straight-chain or branched alkylaldehydes, such as formaldehyde, acetaldehyde, n-propanal, n-butanal, 2-methylpropanal, n-pentanal, 2-methylbutanal, 3-methylbutanal, 2,2-dimethylpropanal, n-hexanal, 2-ethylbutanal, n-heptanal and n-octanal; alkenylaldehydes, such as propenal, 2-methylpropenal, 2-butenal, 2-methyl-2-butenal and 2-ethyl-2-hexenal; cyclic aldehydes, such as cyclopropanecarbaldehyde, cyclopentanecarbaldehyde, cyclopentanecetaldehyde and cyclohexanecarbaldehyde; benzaldehyde, o-, m- and p-toluenecarbaldehyde and phenylacetaldehyde; straight-chain and branched alkylaldehydes which are substituted by hydroxyl, such as 5-hydroxypentanal, 2-hydroxy-3-methylbutanal, 2-hydroxy-2-methylpropanal, 4-hydroxybutanal, 2-hydroxypropanal and 8-hydroxyoctanal; straight-chain and branched alkylaldehydes which are substituted by amino, such as 5-aminopentanal, 2-aminopropanal, 3-aminopropanal, 4-aminobutanal, 2-amino-3-methylbutanal, 8-aminooctanal and mono-N-alkyl derivatives thereof; and straight-chain and branched alkylaldehydes which are disubstituted by amino and hydroxyl, such as 2-hydroxy-5-aminopentanal, 3-hydroxy-3-methyl-4-aminobutanal, 2-hydroxy-4-aminobutanal, 2-hydroxy-3-aminopropanal, 2-hydroxy-2-methyl-3-aminopropanal, 2-amino-3-hydroxyoctanal and mono-N-alkyl derivatives thereof.

Furthermore: methoxy-acetaldehyde, ethoxy-acetaldehyde, n-propoxy-acetaldehyde, i-propoxy-acetaldehyde, n-butoxy-acetaldehyde, i-butoxy-acetaldehyde, tert.-butoxy-

acetaldehyde, cyclopropylmethoxy-acetaldehyde, cyclopropoxy-acetaldehyde, 2-methoxy-ethoxy-acetaldehyde, 2-ethoxy-ethoxy-acetaldehyde, 2-methoxy(1-methyl-ethoxy)-acetaldehyde, 2-ethoxy(1-methyl-ethoxy)-acetaldehyde, phenoxy-acetaldehyde, 2-methoxy-2-methyl-acetaldehyde, 2-ethoxy-2-methyl-acetaldehyde, 2-n-propoxy-2-methyl-acetaldehyde, 2-(i-propoxy-)2-methyl-acetaldehyde, 2-(n-butoxy)-2-methyl-acetaldehyde, 2-(i-butoxy)-2-methyl-acetaldehyde, 2-(tert.-butoxy)-2-methyl-acetaldehyde, 2-cyclopropylmethoxy-2-methyl-acetaldehyde, 2-cyclopropoxy-2-methyl-acetaldehyde, 2-methoxy-ethoxy-α-methyl-acetaldehyde, 2-ethoxy-ethoxy-α-methyl-acetaldehyde, 2-methoxy-(1-methyl-ethoxy)α-methyl-acetaldehyde, 2-methoxy-2,2-dimethyl-acetaldehyde, 2-ethoxy-2,2-dimethyl-acetaldehyde, 2-cyclopropylmethoxy-acetaldehyde, 2-ω-butoxy-2,2-dimethyl-acetaldehyde, methylthio-acetaldehyde, ethylthio-acetaldehyde, n-propylthio-acetaldehyde, i-propylthio-acetaldehyde, cyclopropyl-methylthio-acetaldehyde, 3-methoxy-propanal, 3-ethoxy-propanal, 3-n- and 3-i-propoxy-propanal, 3-n-, 3-i- and 3-tert.-butoxy-propanal, 3-cyclopropoxy-propanal, 3-cyclopropylmethoxy-propanal, 3-methoxy-3-methyl-propanal, 3-ethoxy-3-methyl-propanal, 3-n- and 3-i-propoxy-3-methyl-propanal, 3-n-, 3-i- and 3-tert.-butoxy-3-methyl-propanal, 2,3 and 4-methoxy-butanal, 2,3 and 4-ethoxy-butanal, 2-methylthio-propanal, 2-ethylthio-propanal, 3-methylthio-propanal, 3-ethylthio-propanal, 2-methylthio-butanal, 3-methylthio-butanal, 4-methylthio-butanal, furfurol, tetrahydrofurfurol, thiophene, 5-bromothiophene, 5-methylfurfurol and pyrane-carbaldehyde.

In addition, examples of ketones which may be mentioned are: acetone, methyl ethyl ketone, methyl n-propyl ketone, diethyl ketone, methyl butyl ketone, cyclopentanone, di-n-propyl ketone, cyclohexanone, 3-methylcyclohexanone, 4-methyl-

cyclohexanone, acetophenone, propiophenone, butyrophenone, phenylacetone, p-methoxyacetophenone and m-nitroacetophenone.

Formic acid, for example, can be used as the hydrogen donor reducing agent (Leuckart-Wallach reaction). The formic acid is used in large excess. If formaldehyde is used as the carbonyl component, the reaction can be carried out in aqueous solution, and if ketones and less reactive aldehydes are used, it can be carried out in anhydrous formic acid. The reaction temperatures are between 100 and 200°C, and if appropriate the reaction must be carried out in an autoclave.

Catalytically activated hydrogen can also be used as the hydrogen donor reducing agent. A possible catalyst is, above all, Raney nickel, but noble metal catalysts can also be used. In general, the reaction is carried out under pressures between 80 and 150 atmospheres of H_2 pressure and at temperatures between 70 and 150°C. Preferred solvents are protic, polar solvents, in particular alcohols.

Alkali metal cyanoborohydrides, dialkylaminoboranes and alkali metal borohydrides are also used as hydrogen donor reducing agents. In this process variant, the use of sodium cyanoborohydride is particularly preferred.

In general, the reaction is carried out at room temperature. However, it can also be favourable to heat the mixture briefly to the reflux temperature.

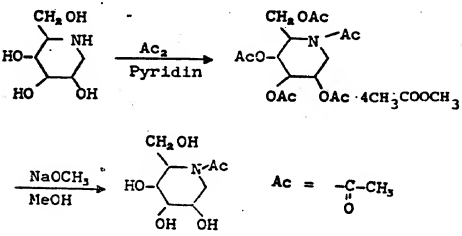
The process is usually carried out in an inert solvent. Although anhydrous aprotic solvents can be employed (for example tetrahydrofurane, if the reducing agent is morpholinoborane), a protic solvent is usually used. A suitable protic solvent is, for example, a lower alkanol or, preferably, water or an aqueous lower alkanol (for example aqueous methanol or ethanol) or other aqueous solvent systems, such as, for

example, aqueous dimethylformamide, aqueous hexamethylphosphoric acid triamide, aqueous tetrahydrofurane or aqueous ethylene glycol dimethyl ether.

The process is usually carried out in a pH range from 1 to 11, and a pH range between 4 and 7 is preferred.

The acid amides of the formula V and urethanes of the formula VI are known in some cases, or they can be obtained by known processes from the compound III and reactive acid derivatives, which can also be formed in situ from the free acids.

In this procedure, the reaction can be carried out in a manner such that only the amino group of the compound III reacts with the acid derivative, for example by using excess acid anhydride in an aqueous or alcoholic solution, or such that the peracylated compounds first form and are then converted into the N-acylated compounds by reaction with alcoholic ammonia or by trans-esterification catalysed by alkali metal alcoholate. The latter process may be illustrated by an example:



The acid amides of the formula II can be reduced to amines of the formula I (R = H) with complex metal hydrides or also with boron hydride compounds. It is preferable to use $NaBH_4$ in pyridine or also sodium acyloxyborohydrides, particularly sodium trifluoroacetoxyborohydride. As a rule, the reducing agents are employed in excess. Sodium trifluoroacetoxyborohydride is produced in situ from sodium borohydride and trifluoroacetic acid. Possible solvents are, in addition to pyridine, polar aprotic solvents, such as dioxane, tetrahydrofurane or diglyme. The reaction is preferably carried out at the boiling point of the solvent. $LiAlH_4$ can also optionally be used for the reduction, preferably when the hydroxyl groups are first protected in the customary way.

The reactive alkylating agents of the formula VII are known or can be prepared by customary processes. The reaction with the compound III is carried out in inert organic solvents at room temperature up to the boiling point, with or without the addition of an acid-binding agent.

In detail, new active compounds which may be mentioned are: N-methyl-l-nojirimycin, N-ethyl-l-nojirimycin, N-n-butyl-l-nojirimycin, N-stearyl-l-nojirimycin, N-i-propyl-l-nojirimycin, N-benzyl-l-nojirimycin, N-allyl-l-nojirimycin, N-(β-methoxyethyl)-l-nojirimycin, N-(β-dimethylaminoethyl)-l-nojirimycin, N-(o-hydroxybenzyl)-l-nojirimycin, N-methyl-l-desoxynojirimycin, N-ethyl-l-desoxynojirimycin, N-n-butyl-l-desoxynojirimycin, N-stearyl-l-desoxynojirimycin, N-i-propyl-l-desoxynojirimycin, N-benzyl-l-desoxynojirimycin, N-allyl-l-desoxynojirimycin, N-(β-methoxyethyl)-l-desoxynojirimycin, N-(β-dimethylaminoethyl)-l-desoxynojirimycin, N-(o-hydroxybenzyl)-l-desoxynojirimycin, N-methyl-l-desoxynojirimycin-l-sulphonic

acid, N-ethyl-1-desoxynojirimycin-1-sulphonic acid, N-n-butyl-1-desoxynojirimycin-1-sulphonic acid, N-stearyl-1-desoxynojirimycin-1-sulphonic acid, N-i-propyl-1-desoxynojirimycin-1-sulphonic acid, N-benzyl-1-desoxynojirimycin-1-sulphonic acid, N-allyl-1-desoxynojirimycin-1-sulphonic acid, N-(β-methoxyethyl)-1-desoxynojirimycin-1-sulphonic acid, N-(β-dimethylaminoethyl)-1-desoxynojirimycin-1-sulphonic acid, N-(o-hydroxybenzyl)-1-desoxynojirimycin-1-sulphonic acid, N-methyl-1-cyano-desoxynojirimycin, N-ethyl-1-cyano-desoxynojirimycin, N-n-butyl-1-cyano-desoxynojirimycin, N-stearyl-1-cyano-desoxynojirimycin, N-i-propyl-1-cyano-desoxynojirimycin, N-benzyl-1-cyano-desoxynojirimycin, N-allyl-1-cyano-desoxynojirimycin, N-(β-methoxyethyl-1-cyano-desoxynojirimycin, N-(β-dimethylaminoethyl)-1-cyano-desoxynojirimycin and N-(o-hydroxybenzyl)-1-cyano-desoxynojirimycin.

The inhibitors according to the invention are suitable for use as therapeutic agents for the following indications: pre-diabetes, gastritis, constipation, caries, atherosclerosis and, in particular, adiposity, diabetes and hyperlipoprotaemia.

In order to broaden the spectrum of activity, it can be advisable to combine inhibitors for glycoside-hydrolases which complement one another in their action, the combinations being either combinations of the inhibitors according to the invention with one another or combinations of the inhibitors according to the invention with inhibitors which are already known. Thus, for example, it can be appropriate to combine saccharase inhibitors according to the invention with amylase inhibitors which are already known.

In some cases, combinations of the inhibitors according to the invention with known oral antidiabetic agents (β-cytotropic sulphonylurea derivatives and/or biguanides having an

action on the blood sugar) and with blood lipid-lowering active compounds, such as, for example, clofibrate, nicotinic acid, cholestyramine and others.

The compounds can be administered without dilution, for example as a powder or in a gelatine casing, or in combination with an excipient in a pharmaceutical composition.

Pharmaceutical formulations can contain a relatively large or relatively small amount of the inhibitor, for example 0.1% to 99.5%, combined with a pharmaceutically acceptable non-toxic, inert excipient, it being possible for the excipient to contain one or more solid, semi-solid or liquid diluents, fillers and/or non-toxic, inert and pharmaceutically acceptable formulation auxiliary. Such pharmaceutical formulations are preferably in the form of dosage units, that is to say physically discrete units containing a particular amount of the inhibitor, which correspond to a fraction or a multiple of the dose which correspond to cause the desired inhibiting action. The dosage units can contain 1, 2, 3, 4 or more individual doses or a ½, 1/3 or 1/4 of an individual dose. An individual dose preferably contains an amount of active compound which is sufficient to achieve the desired inhibiting action on administration according to a previously determined dosage plan of one or more dosage units, a whole, a half or a third or a quarter of the daily dose usually being administered at all the main and secondary mealtimes of the day. Other therapeutic agents can also be taken. Although the dosage and the dosage plan should be carefully considered in each case, applying thorough expert judgement and taking into account the age, the weight and the condition of the patient and the nature and the severity of the disease, the dosage is usually in a range between about 1 to about 1×10^4 SIU/kg of

body weight per day. In some cases a sufficient therapeutic action will be achieved with a relatvely low dose, whilst in other cases a relatively large dose will be required.

Oral administration can be carried out using solid and liquid dosage units, such as, for example, powders, tablets, dragees, capsules, granules, suspensions, solutions and the like.

Powders are prepared by comminuting the substance to a suitable size and mixing it with a pharmaceutical excipient, which has also been comminuted. Although an edible carbohydrate, such as, for example, starch, lactose, sucrose or glucose, is usually used for this purpose and can also be used here, it is desirable to employ a carbohydrate which cannot be metabolised, such as, for example, a cellulose derivative.

Sweeteners, flavouring additives, preservatives, dispersing agents and dyestuffs can also be co-used.

Capsules can be prepared by formulating the powder mixture described above and filling gelatine casings which have already been formed. Lubricants, such as, for example, silica gel, talc, magnesium stearate, calcium stearate or solid polyethylene glycol, can be added to the powder mixture before the filling operation. A disintegrating agent or solubiliser, such as, for example, agar-agar, calcium carbonate or sodium carbonate, can also be added to the mixture in order to improve the accessibility of the inhibitor when the capsule is taken.

Tablets are manufactured, for example, by preparing a powder mixture, coarse or fine grained, and adding a lubricant and disintegrating agent. Tablets are formed from this mixture. A powder mixture is prepared by mixing the substance, which has been comminuted in a suitable manner, and a

diluent or another excipient as to describe above is added. A binder is optionally added: for example carboxymethylcellulose, alginates, gelatine or polyvinylpyrrolidones, and a solution retarder, such as, for example, paraffin, a resorption accelerator, such as, for example, a quaternary salt and/or an adsorbent, such as, for example, bentonite, kaolin or dicalcium phosphate. The powder mixture can be granulated together with a binder, such as, for example, syrup, starch paste or gum acacia, or solutions of cellulose or polymeric materials. Thereafter, the product is pressed through a coarse sieve. Alternatively, it is possible to allow the powder mixture to run through a tablet machine and to comminute the resulting non-uniformly shaped pieces down to the particle size. So that the particles to not jam in the tablet-forming nozzles, a lubricant can be added, such as, for example, stearic acid, a stearate salt, talc or mineral oil. This mixture which has been lubricated is then pressed into tablet form. The active compounds can also be combined with free-flowing inert excipients and brought directly into tablet form, omitting the granulation or comminution steps. The product can be provided with a clear or opaque protective casing, for example a coating of shellac, a coating of sugar or polymeric substances and a polished casing of wax. Dyestuffs can be added to these coatings so that a distinction can be made between the different dosage units.

The formulation forms to be administered orally, such as, for example, solutions, a syrup and elixirs, can be prepared in dosage units so that a particular amount of formulation contains a particular amount of active compound. The syrup can be prepared by dissolving the active compound in an aqueous solution which contains suitable flavouring substances;

elixirs are obtained using non-toxic, alcoholic excipients. Suspensions can be prepared by dispersing the compound in a non-toxic excipient. Solubilisers and emulsifying agents, such as, for example, ethoxylated isostearyl alcohols and polyoxyethylene sorbitol esters, preservatives, and additives which improve the flavour, such as, for example, peppermint oil or saccharin and the like, can also be added.

Dosage instructions can be given on the capsule. Moreover, the dosage can be made safe so that the active compound is released in a delayed manner, for example by holding the active compound in polymeric substances, waxes or the like.

In addition to the abovementioned pharmaceutical compositions, foodstuffs containing these active compounds can also become prepared; for example sugar, bread, potato products, fruit juice, beer, chocolate and other confectionery, and preserves, such as, for example, jam, a therapeutically active amount of at least one of the inhibitors according to the invention having been added to these products.

Furthermore, the inhibitors according to the invention have the property, in animals, of influencing to a high degree the ratio of the proportion of undesired fat to the proportion of desired meat of low fat content (lean meat) in favour of the lean meat. This is of particular importance for the rearing and keeping of agricultural stock animals, for example in the fattening of pigs, but is also of considerable importance for the rearing and keeping of other stock animals and pets. Furthermore, the use of the inhibitors can lead to a considerable rationalisation of the feeding of the animals, both in respect of time, quantity and quality. Since they cause a certain delay in digestion, the residence time of the nutrients in the digestive tract is extended, whereby ad libitum feeding

associated with less expense is made possible. Furthermore, in many cases there is a considerable saving of valuable protein feed when the inhibitors according to the invention are used.

The active compounds can thus be used in virtually all sections of animal nutrition as agents for reducing the formation of fatty layers and for the saving of feed protein.

The activity of the active compounds here is essentially independent of the nature and the sex of the animals. The active compounds prove particularly valuable in species of animals which tend generally to deposit relatively large amounts of fat, or tend to do so during certain stages of their life.

The following stock animals and pets may be mentioned as examples of animals for which the inhibitors for reducing the formation of fatty layers and/or for saving feed protein can be employed: warm-blooded animals, such as cattle, pigs, horses, sheep, goats, cats, dogs, rabbits, fur-bearing animals, for example mink and chinchillas, and other pets, for example guinea pigs and hamsters, laboratory animals and zoo animals, for example rats, mice, monkeys and the like, poultry, for example broilers, chickens, geese, ducks, turkeys and pigeons, parrots and canaries, and cold-blooded animals, such as fish, for example carp, and reptiles, for example snakes.

Because of the favourable properties of the active compounds, the amount of active compounds administered to the animals in order to achieve the desired effect can be substantially varied. It is preferably about 0.5 mg to 2.5 g, in particular 10 to 100 mg/kg, of feed per day. The period over which the active compound is administered can be from a few hours or days to several years. The appropriate amount

of active compound and the appropriate period over which it is administered are closely connected with the object of feeding. In particular, they depend on the nature, the age, the sex and the state of health and the method of keeping the animals and can be easily determined by any expert.

The active compounds according to the invention are administered to the animals by the customary methods. The nature of the administration depends, in particular, on the nature, the behaviour and the general condition of the animals. Thus it is possible to carry out the administration orally once or several times daily, at regular or irregular intervals. In most cases, oral administration, in particular in the rhythm of the food and/or drink intake of the animals, is to be preferred for reasons of expediency.

The active compounds can be administered as pure substances or in the formulated form, the formulated form being understood both as a premix, that is to say mixed with non-toxic inert carriers of any desired nature, and as part of a total ration in the form of a supplementary feed and as a constituent of the mixture of a mixed feed by itself. Administration of suitable formulations by means of the drinking water is also included.

The active compounds, optionally in the formulated form, can also be administered, in a suitable form, together with other nutrients and active compounds, for example mineral salts, trace elements, vitamins, proteins, energy carriers (for example starch, sugar or fats), dyestuffs and/or flavouring substances or other feedstuff additives, such as, for example, growth promoters. The active compounds can be administered to the animals before, during or after the food intake.

Oral administration together with the feed and/or drinking water is advisable, the active compounds being added to the total amount or only to parts of the feed and/or drinking water, depending on the requirement.

The active compounds can be added to the feed and/or the drinking water according to customary methods by simple mixing as the pure substances, preferably in the finely divided form, or in the formulated form mixed with edible, non-toxic carriers, and optionally also in the form of a premix or a feed concentrate.

The feed and/or drinking water can, for example, contain the active compounds according to the invention in a concentration of about 0.001 to 5.0%, in particular 0.02 to 2.0% (weight). The optimum level of the concentration of the active compound in the feed and/or drinking water depends, in particular, on the amount of feed and/or drinking water intake of the animals and can be easily determined by any expert.

The nature of the feed and its composition is not important here. It is possible to use all the current, commercially available or special feed compositions, which preferably contain the customary balance of energy substances and proteins, including vitamins and mineral substances, necessary for balanced nutrition. The feed can be composed, for example, of vegetable substances, for example shredded oil-cake, shredded cereal and cereal by-products, but also of hay, silage fodder, beets, and other forage plants, of animal substances, for example meat and fish products, bonemeal, fats and vitamins, for example A, D, E, K and B-complex, as well as special sources of protein, for example yeasts and certain aminoacids, and mineral substances and trace elements, such as,

for example, phosphorus and iron, zinc, manganese, copper, cobalt, iodine and the like.

Premixes can preferably contain about 0.1 to 50%, in particular 0.5 to 5.0% (weight) of, for example, N-methyl-1-desoxynojirimycin, in addition to any desired edible carriers and/or mineral salts, for example carbonated feed lime, and are prepared by the customary mixing methods.

Mixed feeds preferably contain 0.001 to 5.0%, in particular 0.02 to 2.0% (weight), for example, of N-methyl-1-desoxynojirimycin, in addition to the customary raw material components of a mixed feed, for example shredded cereal or cereal by-products, shredded oilcake, animal protein, minerals, trace elements and vitamins. They can be prepared by the customary mixing methods.

The active compounds in premixes and mixed feedstuffs can preferably also be appropriately protected from air, light and/or moisture by suitable agents which cover their surface, for example with non-toxic waxes or gelatine.

The following is an example of the composition of a finished mixed feed, for poultry, containing an active compound according to the invention: 200 g of wheat, 340 g of maize, 360.3 g of coarse soya bean meal, 60 g of beef tallow, 15 g of dicalcium phosphate, 10 g of calcium carbonate, 4 g of iodinated sodium chloride, 7.5 g of a vitamin/mineral mixture and 3.2 g of an active compound premix give, after careful mixing, 1 kg of feed.

The vitamin/mineral mixture consists of: 6,000 I.U. of vitamin A, 1,000 I.U. of vitamin D_3, 10 mg of vitamin E, 1 mg of vitamin K_3, 3 mg of riboflavin, 2 mg of pyridoxine, 20 mcg of vitamin B_{12}, 5 mg of calcium pantothenate, 30 mg of nicotinic acid, 200 mg of choline chloride, 200 mg of

$MnSO_4 \times H_2O$, 140 mg of $ZnSO_4 \times 7H_2O$, 100 mg of $FeSO_4 \times 7H_2O$ and 20 mg of $CuSO_4 \times 5H_2O$. The active compound premix contains, for example, 1-desoxynojirimycin in the desired amount, for example 1,600 mg, and in addition 1 g of DL-methionine and enough soya bean flour to form 3.2 g of premix.

The following is an example of the composition of a mixed feed, for pigs, which contains an active compound of the formula I: 630 g of shredded cereal feed (composed of 200 g of shredded maize, 150 g of shredded barley, 150 g of shredded oats and 130 g of shredded wheat), 80 g of fishmeal, 60 g of coarse soya bean meal, 58.8 g of tapioca flour, 38 g of brewer's yeast, 50 g of a vitamin/mineral mixture for pigs (composition, for example, as in the chicken feed), 30 g of linseed cake meal, 30 g of maize gluten feed, 10 g of soya bean oil, 10 g of cane sugar molasses and 2 g of active compound premix (composition, for example, as in the chicken feed) give, after careful mixing, 1 kg of feed.

The feed mixtures indicated are intended, preferably, for rearing and fattening chicken or pigs respectively; however, they can also be used in an identical or similar composition for rearing and fattening other animals.

The inhibitors can be used individually or also in any desired mixtures with one another.

In vitro saccharase inhibition test

The in vitro saccharase inhibition test makes it possible to determine the enzyme-inhibitory activity of a substance by comparison of the activity of the solubilised intestinal disaccharidase complex in the presence and in the absence (so-called 100% value) of the inhibitor. A virtually glucose-free sucrose (glucose $<$ 100 ppm) is used here as the substrate which determines the specificity of the

inhibition test; the determination of enzyme activity is based on the spectrophotometric determination of glucose liberated, using glucose dehydrogenase and nicotinamide-adenine dinucleotide as the cofactor.

One saccharase inhibitor unit (SIU) is defined as that inhibitory activity which, in a defined test batch, reduces a given saccharolytic activity by one unit (saccharase unit = SU); the saccharase unit is defined here as that enzyme activity which splits off one µmol of sucrose per minute under given conditions and thus leads to the liberation of one µmol each of glucose, which is determined in the test, and fructose, which is not recorded in the test.

The intestinal disaccharidase complex is obtained from swine small intestine mucosa by tryptic digestion, precipitation from 66% strength ethanol at -20°C, taking up of the precipitate in 100 mM phosphate buffer, pH 7.0, and final dialysis against the same buffer.

100 µl of a dilution of the intestinal disaccharidase complex in 0.1 M maleate buffer, pH 6.25, are added to 10 µl of a sample solution, which is prepared such that the extinction of the test batch is at least 10%, but not more than 25%, below that of the 100% value, and the mixture is pre-incubated at 37°C for 10 minutes. The dilution of the disaccharidase complex should be adjusted to an activity of 0.1 SU/ml.

The saccharolytic reaction is started by adding 100 µl of a 0.4 M solution of sucrose ("SERVA 35579") in 0.1 M maleate buffer, pH 6.25, and, after an incubation period of 20 minutes at 37°C, is stopped by adding 1 ml of glucose dehydrogenase reagent (1 small bottle of lyophilised glucose dehydrogenase/mutarotase mixture ("MERCK 14053") and 331.7 mg of β-nicotinamide-adenine dinucleotide (free acid, "BOEHRINGER" degree of

purity I) dissolved in 250 ml of 0.5 M tris buffer, pH 7.6). In order to determine the glucose, the mixture is incubated at 37°C for 30 minutes and finally is measured photometrically at 340 nm against a reagent blank (containing enzyme but without sucrose).

The calculation of the inhibitory activity of inhibitors is made difficult by the fact that even slight changes in the test system, for example a 100% value which varies slightly from determination to determination, have an effect on the test result which can no longer be ignored. These difficulties are avoided by running a standard with every determination; a saccharase inhibitor of the formula $C_{25}H_{43}O_{18}N$ which has a specific inhibitory activity of 77,700 SIU/g and, when employed in the test in amounts of 10 to 20 ng, leads to an inhibition of the order of size specified above, is used as the standard. If the difference between the extinctions at 340 nm of the 100% value and of the batch inhibited by the standard is known, the specific inhibitory activity of the inhibitor, expressed in saccharase inhibitor units per gram (SIU/g), can be calculated in a known manner from the extinction difference between the 100% value and the batch inhibited by the sample solution, taking into consideration the amount of inhibitor employed.

Specific saccharase-inhibitory activity in vitro

1-Desoxynojirimycin	465,000 SIU/g
N-Methyl-1-desoxynojirimycin	2,330,000 SIU/g

Preparation Example

3.2 g of 1-desoxynojirimycin and 2 ml of 30% strength aqueous formaldehyde are added to 4 ml of 98% strength formic acid, whilst cooling with ice. The mixture is then heated under reflux for 8 hours. After cooling, the reaction mixture is diluted with acetone. A resinous precipitate

separates out. The acetone solution is decanted off and the resin is rinsed several times with acetone. The residue is then dissolved in distilled water and the solution is freed from formic acid by adding a basic ion exchanger in the $^{\ominus}OH$ form (Amberlite JRA 410). The ion exchanger is filtered off and the aqueous solution is brought to dryness under reduced pressure. 3.0 g of resinous N-methyl-1-desoxy-nojirimycin remain. The compound can be further purified by chromatography on cellulose. Water-containing butanol is used as the running agent.

For characterisation, the compound is converted into the peracetylated compound, N-methyl-2,3,4,6-tetra-O-acetyl-1-deoxynojirimycin, with acetic anhydride/pyridine 1:1 at room temperature. A proton magnetic resonance spectrum of this derivative in $CDCl_3$ was measured at 100 MHz: 4 singlets for the total of 12 protons, which correspond to the methyl groups of the O-acetyl groups ($\underline{CH_3}$-O-C-), are found between δ = 2.0 and 2.1 ppm. The methyl group bonded to N ($\underline{CH_3}$-N<) is found as a singlet at δ = 2.45 ppm. Two protons on a C atom bonded to nitrogen (-C-N<) absorb as poorly resolved multiplets between δ = 2.1 and 2.5 ppm. A further proton of this type appears as a doublet of a doublet (J_1 = 11 Hz; J_2 = 4 Hz) at δ = 3.18 ppm. A methylene group ($-\underline{CH_2}$-O-C-CH_3) absorbs as an AB system at δ = 4.16 and δ = 4.22 ppm. The remaining three protons (-C(H)-O-C-CH_3) are found as a multiplet between δ = 4.9 and 5.2 ppm.